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Identification of Differential Protein Expression in Response to the Application of BioRegulators that Enhance Plant Productivity and Quality

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The enhancement of plant growth and productivity by the use of chemicals is a common practice in agriculture but rather little is known about the effect these BioRegulators have on plants on a molecular level. The presented work is part of an ERA-NET project with the aim to identify genes, proteins and metabolites, differentially expressed under conditions of stress while treated with certain compounds. Arabidopsis thaliana plants are grown on soil and in hydroponic cultures and are subjected to salt, cold and drought stress. The progress of stress is monitored phenotypically by leaf shape and chlorophyll fluorescence. The proteome profile of the plants is analysed via 2D electrophoresis and mass spectrometry. For the identification of differentially regulated proteins in response to the BioRegulator treatment under stress conditions, the DIGE (difference-in-gel-electrophoresis) system will be used. The biomarkers identified universally for the different stress conditions will be used to establish a cell based assay for the screening of potential new BioRegulator compounds.

1 Plant Growth

Arabidopsis plants are grown on soil for the study of cold stress and in hydroponic cultures for the study of salt and drought stress. In both setups plants are grown for three weeks under short day conditions (9 hrs light, 15 hrs dark) and one week under long day conditions (16 hrs light, 8 hrs dark) before they are subjected to the respective stress conditions. For cold stress conditions the plants are shifted to a day/night temperature regime of 4 °C. Control plants stay at the day/night temperature regime of 21°C/18°C. Salt stress is produced by supplementing the hydroponic solutions with 125 mM NaCl and drought stress by supplementing the solution with 20 % PEG, thereby lowering the water potential.

2 Stress Monitoring

Stress monitors had to be found in order to detect significant changes of plant behaviour under stress conditions while treated with BioRegulator compounds. For cold stress the decreasing quantum yield of photosynthesis (probed via chlorophyll fluorescence of PSII) can be used as a reliable indicator for stress. Healthy plants exhibit a ratio of 0.8 whereas a decrease below 0.7 indicates disturbance and damage of the photosynthetic apparatus by the stress. The onset of salt stress is monitored in changes of leaf shape as in comparison to control plants, the leaves of stressed plants grow rather in width than in length. The ratio between leaf length and width was measured at certain time points during a salt stress period of 144 hrs. At the end the ratios were 2.22 ± 0.14 and 2.78 ± 0.24 for stressed and control plants respectively. Drought stress leads to a reduced overall growth and a

darker green colouring compared to normal conditions but these parameters are so far only documented photographically. Using the accumulation of anthocyanin, a purple coloured secondary metabolite as a stress monitor is currently tested.

3 Compound Treatment

BioRegulator compounds are sprayed onto the plants 24 hrs before they are shifted to the respective stress conditions. Among others, compounds from the groups of azoles and neonicotinoids are used in the study.

4 Proteomic Analysis

2D electrophoresis is used for the analysis of the whole leaf proteome. Proteins extracted from Arabidopsis leaves are separated according to their isoelectric point (pI) in the first dimension by isoelectric focusing (IEF) and according to their molecular weight in the second dimension by SDS polyacrylamid gel electrophoresis (PAGE). For the identification of the biomarkers 2D electrophoresis using fluorescent protein tags (DIGE, difference in gel electrophoresis) is used for the identification of the biomarkers. With this method, two samples from different conditions to be compared are labelled with fluorescent dyes that are spectroscopically distinguishable (CyDyesTM GE Healthcare) and run on the same 2D gel. An internal standard consisting of a pool of all samples present in the experiment is run on every gel in order to facilitate inter-gel matching and statistic evaluation (three samples per gel, CyDyes 2, 3 and 5). Spot intensities from the different images are obtained and protein difference ratios are determined using the DeCyder software (GE Healthcare).

First the difference in protein composition in response to cold stress conditions untreated with BioRegulators was investigated by DIGE. Arabidopsis plants were subjected to cold stress for a period of 144 hrs and leaf samples (in triplicates) were taken every 24 hrs. In total 8 gels were run. After 144 hrs of cold stress a total of 112 protein spots were found to be differently regulated. Of these, 64 spots increased and 48 spots decreased in abundance. In total ~ 1300 spots were detected on the 2D map of the whole leaf proteome. Protein identification will be done by LC-MS.

In the next steps plants showing a significant effect when treated with BioRegulator compounds under stress conditions (monitored by the parameters introduced in part 2) will be investigated by DIGE in order to indentify the biomarkers necessary for the establishment of the screening assay.

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